Gilbert Spring Study
Macroinvertebrate Community Assessment
By
Faron D. Usrey
Buffalo National River

Introduction

The Buffalo National River (BUFF) has been collecting water-quality on spring systems for more than a decade, and subsequent analysis has shown that Gilbert Spring is highest in average nitrate concentration among the spring systems (Mott, 1997). The town of Gilbert, Arkansas is located above and adjacent to Gilbert Springs, and has no formal sewage treatment facilities. Disposal of raw, household sewage is through on-site septic systems. Since the town of Gilbert is located over a karst network, and is in close proximity to Gilbert Spring, septic leachate was suspected to be a factor in the high nutrient concentrations observed at Gilbert Spring.

In most aquatic systems, biological uptake and assimilation of nutrients into organisms is accomplished primarily by autotrophs such as aquatic macrophytes, phytoplankton, and epilithic periphyton. Assimilation rates of dissolved nutrients by periphyton are influenced by the availability of the nutrients and physical environmental parameters such as light, space, stream velocity, and water temperature. The aquatic community response to a moderate increase in nutrients typically includes an increase in periphytic density followed by shifts in macroinvertebrate community structure (Allan, 1996).

This section will investigate possible differences between the Gilbert Spring's macroinvertebrate community as compared to another system with lower nutrient concentrations, Mitch Hill Spring. Both spring systems have background information on water-quality and to some extent, the macroinvertebrate communities. Given the high nutrients at Gilbert Spring, the logical prediction is that differences in macroinvertebrate communities between the two communities could be explained by the excess nutrients at Gilbert Spring. The goals of this investigation are to determine if there are differences between the macroinvertebrate communities, and to provide possible explanations as to why the macroinvertebrate communities may differ in structure.

Methods

Seasonal variation within macroinvertebrate communities can be large (Barbour et. al., 1999); therefore, both hypocrenal (springbrook extending away from source) systems were sampled seasonally. Spring was considered between March through May, summer was June through August, fall was September through November, and winter was December through February. The discharges from the both spring systems were large compared to other systems within the Buffalo River watershed (Mott, 1997), and fluvial hydraulic habitats could be distinguished below the discharge sources. Of the hydraulic habitats observed, riffle habitats were chosen to represent the habitat for system comparisons. Riffle habitats are known to contain the greatest macroinvertebrate community diversity among fluvial geomorphic habitats. They are preferred as the habitat most suitable for macroinvertebrate community comparison studies (Plafkin et. al., 1989, and Doisy and Rabeni, 1999). Relationships between macroinvertebrate taxa and their respective physical habitats are important in determining differences between sites and quantitative sampling at points within the riffle habitat preserves, as closely as possible, macroinvertebrate taxa dependencies upon physical habitat parameters. For this reason, a quantitative Hess sampler was used to preserve the faunal dependence upon the physical habitat gradients.

Hess sampling was conducted at three locations within each of the first three riffle habitats below the springs. The riffle habitat was divided into ten equidistal length and width axis points. The ten points were numbered 1 through 10, and a random selection was performed for both length and width axis producing 1 sampling point from within the riffle habitat. This was done 3 times for each of the 3 riffles sampled. This resulted in a sampling size of 9 per spring system per season, and a total of 36 samples per site, per year. Once the sample point was selected, the site was marked with an anchored buoy; each buoy was marked with the riffle number and sample number. The riffle closest to the source was labeled riffle number 1, and the top most sampling point was sample 1(i.e. R1-S1 represents the sample closest to the source). At these buoys, water depth, water velocity, and canopy density was measured. Care was taken to stay below all sample locations during the initial point collections. Once these measurements were taken, the sampler was placed into the substrate to the depth of approximately 10-cm. Prior to the active organism collection, 40 substrate particles were randomly selected, washed into the collection net and measured using calipers. The 40 particles were used to produce substrate size means and composition diversities. Once the interior substrates were measured, the remaining contents were stirred for 3 minutes and the suspended organisms were washed into the collection bucket. Samples were placed into containers with a 70% Ethanol preservative. Sample containers were labeled inside and out with site, date, riffle and sample number.

Sample processing was conducted within the laboratory. Macroinvertebrate samples were individually placed into a white picking pan that was divided into 10 sections. The sample was homogenized and organisms within a randomly chosen section were removed. Due to the large number of organisms within each sample, subsampling was conducted. In order to determine the number of organisms needed to represent a sample, three samples from each of the systems were processed in allocates of 100 organisms until the entire sample was processed. Adequate levels of organism counts were determined, and organisms collected from the subsampling were grouped by taxa and identified to the lowest practical taxonomic level. The genus level was achieved for most taxa groups. Numerous national and regional keys were used in identifying the macroinvertebrate taxa (Bednarik and McCafferty 1979, Kondratieff and Voshell 1984, Merritt and Cummins 1996, Pennak 1989, Pflieger 1994, Poulton and Stewart 1991, Provonsha 1990, Stewart and Stark 1993, and Wiggins 1998).

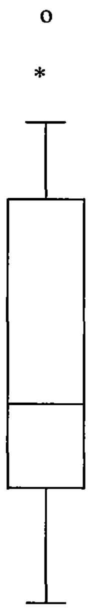
The macroinvertebrate community was characterized using taxa richness, Simpson's index of diversity, percent Ephemeroptera/Plecoptera/Trichoptera (EPT), percent Diptera, and a multimetric approach especially designed for the macroinvertebrate communities of the Buffalo River watershed (Mathis, 2001). Individual metrics was used to evaluate the community's responses to various gradients in physical habitat and water-quality. Taxa richness, Simpson's diversity, and percent EPT are expected to be reduced by a general increase in perturbation, and percent Diptera is expected to increase with increasing perturbation (Barbour et. al., 1999). An

Index of Community Integrity (ICI) was also used to evaluate the overall conditions of the macroinvertebrate communities. The ICI uses 10 common indices, which are known to react to perturbation in predicable a manner. The following indices are used in the generation of the ICI score: Margalef's Index of Taxa Richness, Shannon's Taxa Diversity Index, Percent Dominant Taxa, Percent Chironomidae, Percent Plecoptera, Percent Trichoptera, Percent Elmidae, Percent *Corbicula*, Percent Intolerant, and Percent Collector-Filterer. By adding together all the normalized scores for the metrics calculated on each site, a total ICI score was generated.

Water-quality measurements used in comparing two spring systems were taken from the NPS-BUFF water-quality database. A total of 138 records from 1990 until the fall of 2000 were divided into seasonal categories, and seasonal means were used in correlations with macroinvertebrate community metrics. Nitrates (NO_3) and orthophosphates (PO_4) were the nutrients of interest, and were selected to use in site comparisons.

Two-way Analysis of Variance (ANOVA) was used to determine differences between the two spring systems for the macroinvertebrate communities. The dependent variables for the macroinvertebrate communities were the community metrics previously mentioned. Two-way ANOVA was selected over a T-test because two effect categories (factors) were required to compare the spring communities, and possible interactions between categories were expected (site, season, and site*season). Potential associations between physical habitat and macroinvertebrate community metrics were generated using a Pearson's correlation matrix with a Bonferroni's post hoc test for significance, which accounts for pair-wise alpha inflation. Potential relationships between macroinvertebrate community indices and water-quality parameters were tested using a Spearman's rank correlation; however, with the small sample size a perfect correlative value was required before achieving significance. All assumptions for ANOVA, Pearson's, and Spearman's correlations were checked before the analyses were performed (Berk 1994, and Sokal and Rolf 1995).

Graphs that represented the variation within and among habitats and sites used box plots that are different from traditional box plots (Figure 1). The length of each box shows the range within which the central 50% of values fall, with the box hinges (borders) at the first and third quantiles. The whiskers show the range of values that fall within the inner fences (but do not necessarily extend all the way to the inner fences). Values between the inner and outer fences are plotted with asterisks, and values outside the outer fence are plotted with empty circles (Systat, Version 8).



Explanation of the Box Plot Distributions

Box Plots divide the data into four equal parts. The three borders separating the four parts are called the first, second, and third quartiles. ¼ of the data are to the left of the first quartile, ½ are to the left of the second quartile, and ¾ are to the left of the third quartile. The 2^{nd} quartile is also the median. The length of each box shows the range within which the central 50% of the values fall.

Whiskers are the lines extending out from the main box. These indicate the upper and lower ranges of the data, but the whisker lengths are limited to no more than 50% of the box length. Whiskers also show the range of values that fall within the inner fences.

Hinges are values that fall at the beginning and end of the box length, the end of the 1^{st} and 3^{rd} quartile.

Inner Fences are the values that falls outside of the adjacental hinge.

Outer Fences are the values that fall at the extreme of the whisker, constrained by the 50% box length limit.

Lower inner fence = lower hinge (median lower hinge)
Upper inner fence = upper hinge + (1.5 (upper hinge median))
Lower outer fence = lower hinge (3 (median lower hinge))
Upper outer fence = upper hinge + (3 (upper hinge median))

Asterisks are plotted when value fall between the inner and outer fences.

Empty Circles indicate values outside the outer fences.

Figure 1. Explanation of Box Plot distributions by quartiles, whiskers, and outside values as presented within this investigation. Box Plots generally show within site variation for between site visual comparisons.

Results

A total of 12,830 macroinvertebrates were processed, sorted and identified resulting in 4 Phylum, 4 Classes, 10 Orders, 28 Families, and 33 individual taxa (Table 1). Mean macroinvertebrate taxa richness was highest at Gilbert Spring throughout all the seasons (Figure 2, Table 2). Mean macroinvertebrate diversity (Simpson's Index) was higher at Mitch Hill Spring throughout the seasons (Figure 3, Table 2). During spring, fall, and winter, Gilbert Spring exhibited higher % EPT than did Mitch Hill Spring (Figure 4, Table 2). Gilbert Spring had higher percentages of *Cheumatopsyche* and *Agapetus*; two members of the order Trichoptera than did Mitch Hill Spring. Mean % Diptera was also higher in Mitch Hill Spring for all seasons (Figure 5, Table 2). *Gammarus minus* (a.k.a. sideswimmer or scud) was the dominant organism in both of the hypocrenal systems. Although, Mitch Hill Spring had approximately 1.5 times more *Gammarus minus* than Gilbert Springs. ICI seasonal averages were consistently higher at Mitch Hill Spring for all seasons (Figure 8, Table 2).

Table 1. Taxonomic list of macroinvertebrates collected from Mitch Hill Spring and Gilbert Spring.

Phylum	Class	Order	Family	Genus and species
Annelida	Oligochaeta			
Arthopoda	Crustacea	Amphipoda	Gammaridae	Gammarus minus
Arthopoda	Crustacea	Isopoda	Asellidae	Lirceus
Arthopoda	Insecta	Coleoptera	Gyrinidae	Dineutus
Arthopoda	Insecta	Coleoptera	Elmidae	Optioservus
Arthopoda	Insecta	Coleoptera	Elmidae	Psphenus
Arthopoda	Insecta	Crustacea	Cambaridae	Orconectes
Arthopoda	Insecta	Diptera	Chironomidae	
Arthopoda	Insecta	Diptera	Empididae	Hemerodromia
Arthopoda	Insecta	Diptera	Elmidae	Ordobrevia
Arthopoda	Insecta	Diptera	Simullidae	
Arthopoda	Insecta	Diptera	Tipulidae	Tipula
Arthopoda	Insecta	Ephemeroptera	Beatidae	Beatis
Arthopoda	Insecta	Ephemeroptera	Isonychiidae	Isonychia bicolor
Arthopoda	Insecta	Ephemeroptera	Lepidostomatidae	Lepidostoma
Arthopoda	Insecta	Ephemeroptera	Leptophlebiidae	Leptophlebia
Arthopoda	Insecta	Ephemeroptera	Heptageniidae	Stenacron
Arthopoda	Insecta	Ephemeroptera	Heptageniidae	Stenonema femoratum
Arthopoda	Insecta	Meloptera	Chauliodinae	Nigronia
Arthopoda	Insecta	Plecoptera	Agrionidae	Calopteryx
Arthopoda	Insecta	Plecoptera	Uenoidae	Neophylax fuscus
Arthopoda	Insecta	Plecoptera	Perlidae	Perlesta
Arthopoda	Insecta	Plecoptera		
Arthopoda	Insecta	Trichoptera	Glossosomatidae	Agapetus
Arthopoda	Insecta	Trichoptera	Hydropsychidae	Cheumatopsyche
Arthopoda	Insecta	Trichoptera	Philopotamidae	Chimarra
Arthopoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes
Arthopoda	Insecta	Trichoptera	Helicopsychidae	Helicopsyche
Arthopoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche scalaris
Arthopoda	Insecta	Trichoptera	Psychomyiidae	Psychomyia
Arthopoda	Insecta	Trichoptera	Limnephilidae	Pycnopsyche
Arthopoda	Insecta	Trichoptera	Hydroptilidae	Stacobiella
Mollusca	Bivalvia	Pelecypoda	Corbiculidae	Corbicula fluminea
Mollusca	Gastropoda			
Nematomorpha				
Tubellaria				

Table 2. Seasonal means of macroinvertebrate community metrics from Mitch Hill Spring (S33) and Gilbert Spring (S41).

Site	Season	Substrate Diversity	Substrate Average	Taxa Richness	Taxa Diversity	% EPT	%Diptera	%Gammarus	%Agapetus	ICI
S33	Spring	0.381	36.3	5.4	0.535	6.8	9.3	66.8	6.2	44
S33	Summer	0.371	41.8	6.1	0.523	13.6	17.4	66.3	0.7	45.3
S33	Fall	0.417	38.4	5.2	0.756	6.8	4.2	86.4	1.3	44.7
S33	Winter	0.388	36.5	7.1	0.604	13.1	6.9	76.1	4.0	44.7
S41	Spring	0.444	28.8	9.1	0.350	12.9	2.4	49.1	6.8	35.3
S41	Summer	0.393	18.4	6.9	0.404	6.4	1.0	40.5	5.0	40.7
S41	Fall	0.410	24.0	8.9	0.330	43.6	2.4	43.5	14.9	38
S41	Winter	0.499	18.1	8.6	0.379	18.8	1.7	53.9	11.6	42

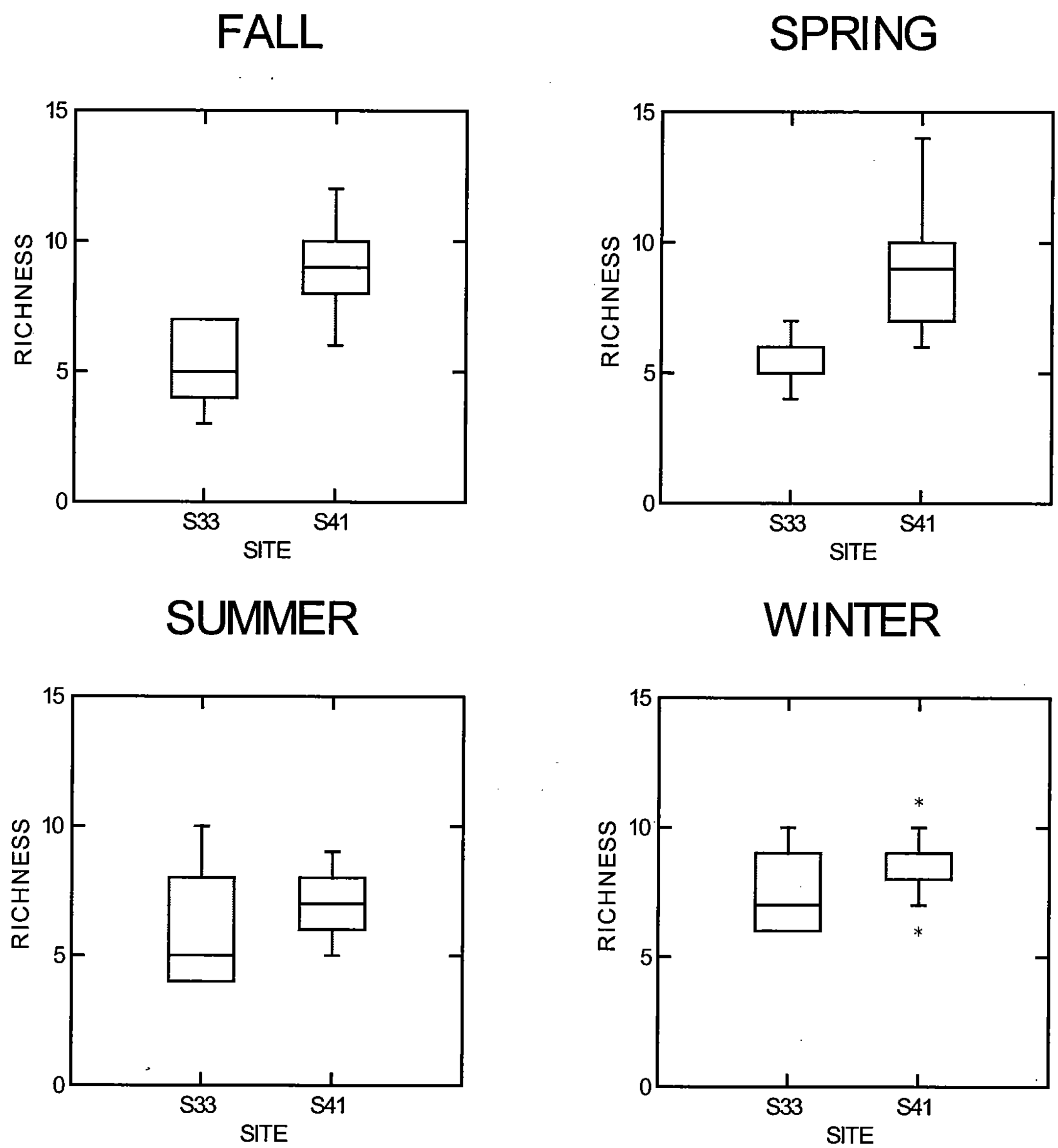


Figure 2. Macroinvertebrate taxa richness comparison between Gilbert Spring (S41) and Mitch Hill Spring (S33) for four seasons (2000/2001).

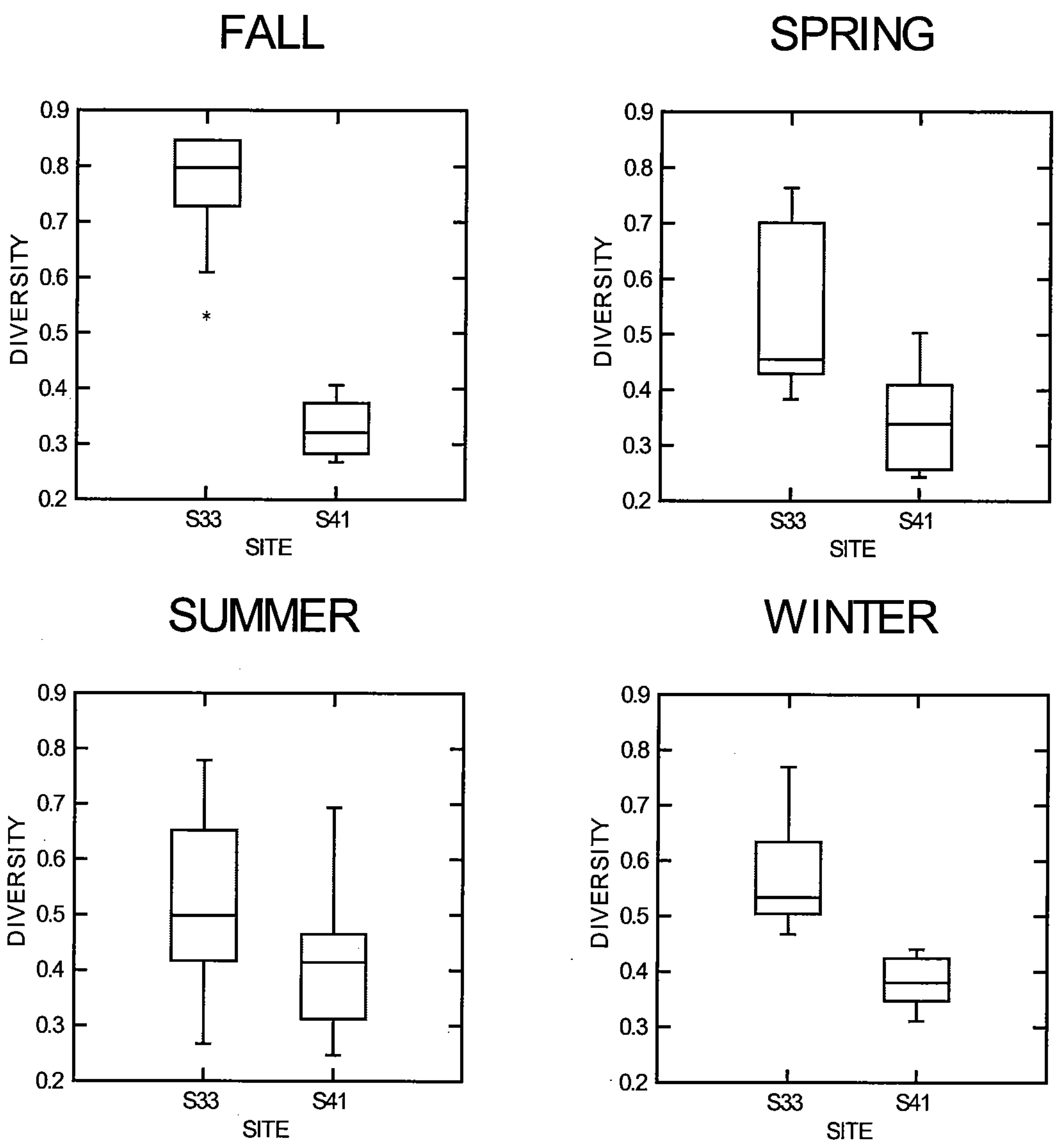


Figure 3. Macroinvertebrate community diversity (Simpson's Index) for Gilbert Spring (S41) and Mitch Hill Spring (S33) for four seasons.

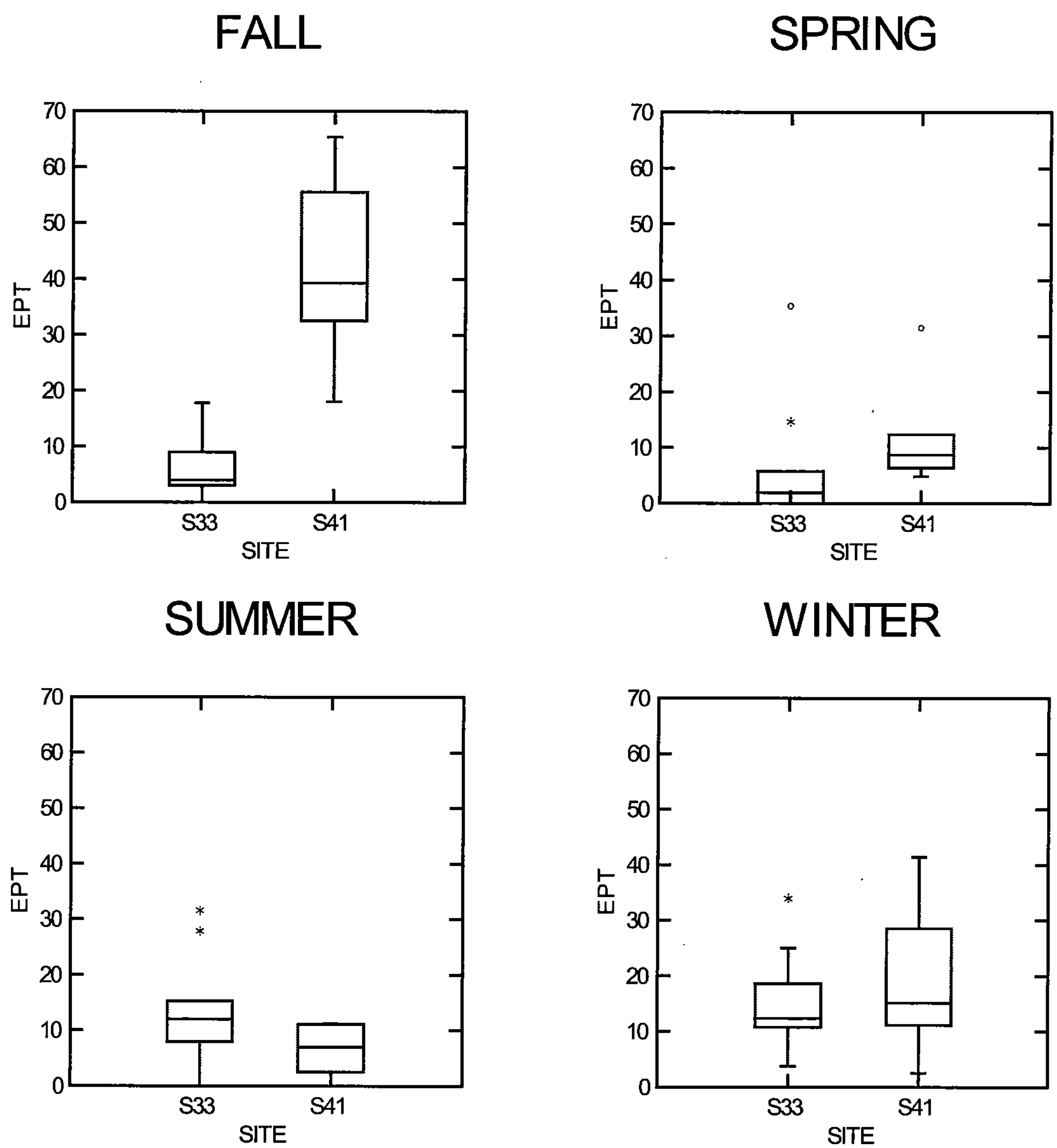


Figure 4. Percent Ephemeroptera/Plecoptera/Trichoptera (EPT) at Gilbert Spring (S41) versus Mitch Hill Spring (S33) during four seasons.

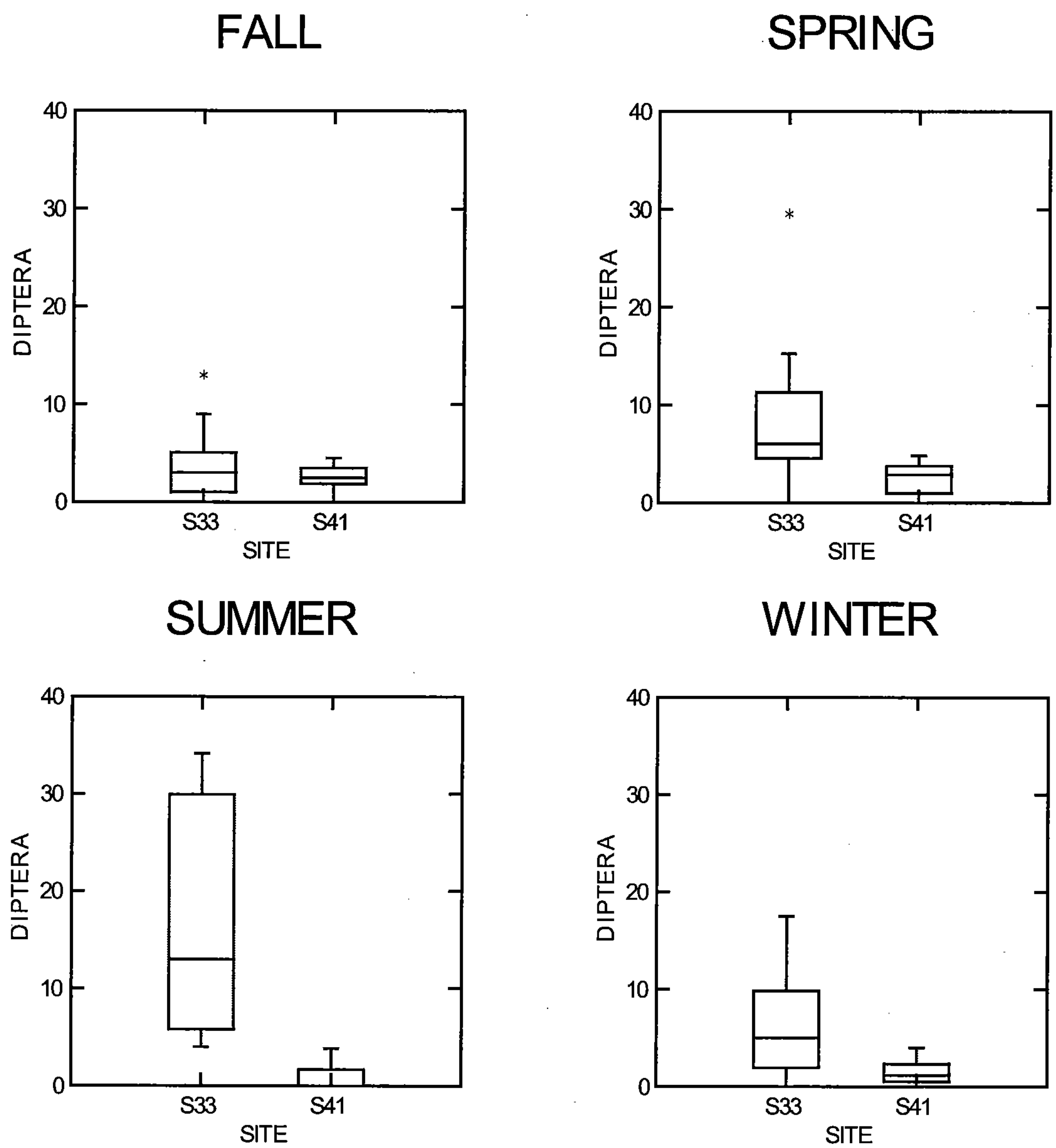


Figure 5. Percent Diptera at Gilbert Spring (S41) versus Mitch Hill Spring (S33) for four seasons.

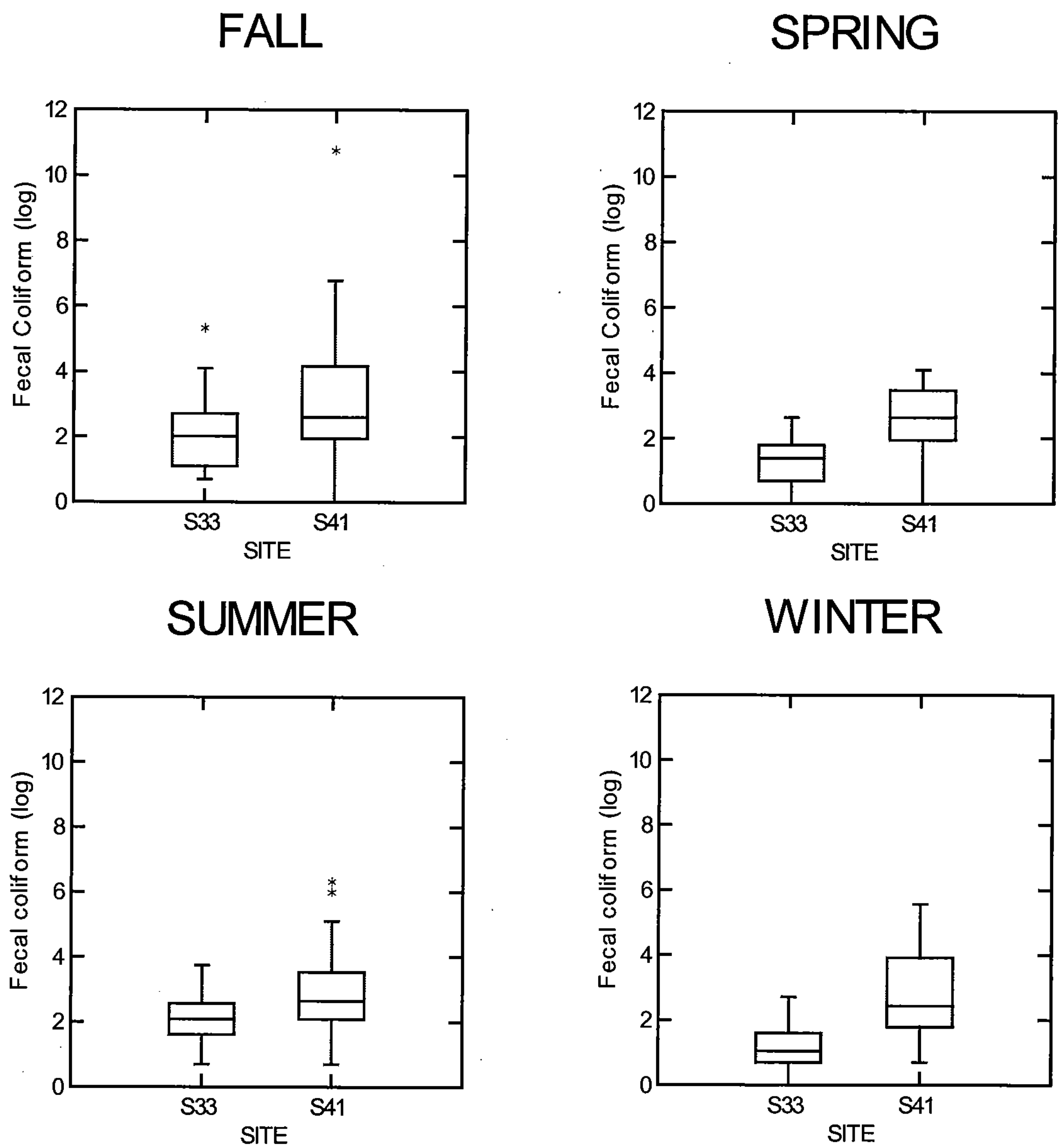


Figure 6. Fecal Coliform means by season from Gilbert Spring (S41) and Mitch Hill Spring (S33).

Physicochemical characteristics of mean temperature, conductivity (μmhos), pH, dissolved oxygen, and turbidity (NTU) were similar between the spring systems, with Gilbert Spring being slightly higher in turbidity (Table 3). Mean fecal coliform density and nitrates concentrations (NO3) were higher at Gilbert Spring for all seasons (Figure 7, Table 3). Mean orthophosphate concentrations were highest at Gilbert Spring for summer, fall, and winter. No relationships between nitrates and orthophosphates were found with macroinvertebrate community indices (seasonal mean comparison, n = 4 per system).

The physical characteristics of the two spring systems are quite dissimilar. The tail waters that create the fluvial habitat at Gilbert Springs are quite short in length, approximately 530 feet. The system at Gilbert Springs is totally contained within the Buffalo River flood plain, and subject to seasonal, backwater flooding from the Buffalo River. Alternatively, Mitch Hill Spring is located approximately 1/8th of a mile from the Buffalo River, and well above the river's flood plain. Mitch Hill is also bordered on the west by a gravel road that extends nearly the entire length of fluvial system. Mitch Hill was deeper during all seasons sampled and had higher velocities, with the exception of spring season (Table 4). Gilbert Spring was considerably higher in mean percent canopy coverage for all seasons (Table 4). Substrate sizes were typically larger at Mitch Hill, but substrate diversity was higher at Gilbert Spring, with the exception of fall (Table 4). After the spring sampling and prior to summer sampling, a large flood event occurred within the Buffalo River watershed. Based on visual observations of effect after the flood, Mitch Hill Spring had lost most of the attached vegetation, and the vegetation was displaced downstream. Gilbert Spring was inundated with fine sediment and organics, as a result of its location within the flood plain of the Buffalo River.

Two-way analysis of variance (ANOVA) resulted in the macroinvertebrate communities being significantly different for the metrics of % Diptera, % Gammarus, % Agapetus, and ICI (Bonferroni's post hoc test for significance, Table 5). The two categorical factors used in the analysis were "sites" and "season". Interactions between the factors of "site" and "season" were significant for three of the community indices; Taxa Richness, Species Diversity, and % EPT (p-values: 0.024, 0.001, and 0.000, respectively, Table 5). The interaction of site and season for these indices indicate that the effect of season is an integral part of the effect of site; therefore, the determination of difference cannot be made for the effect of sites independent of the effect of season. All dependent variables used in the two-way ANOVA were examined for independence, constant variance, and normality.

Pearson's test for correlation found no relationships between physical habitat measurements and macroinvertebrate community indices at Mitch Hill Spring. Gilbert Spring's macroinvertebrate community had taxa richness positively related to bottom velocity ($r = 0.491$, p-value 0.024, $n = 36$) and to substrate size ($r = 0.532$, p-value 0.008, $n = 36$). Correlative coefficients were generated at the highest scale, the two systems combined ($n = 72$), and macroinvertebrate diversity was positively related to depth ($r = 0.503$, p-value 0.000) and substrate size ($r = 0.416$, p-value 0.003).

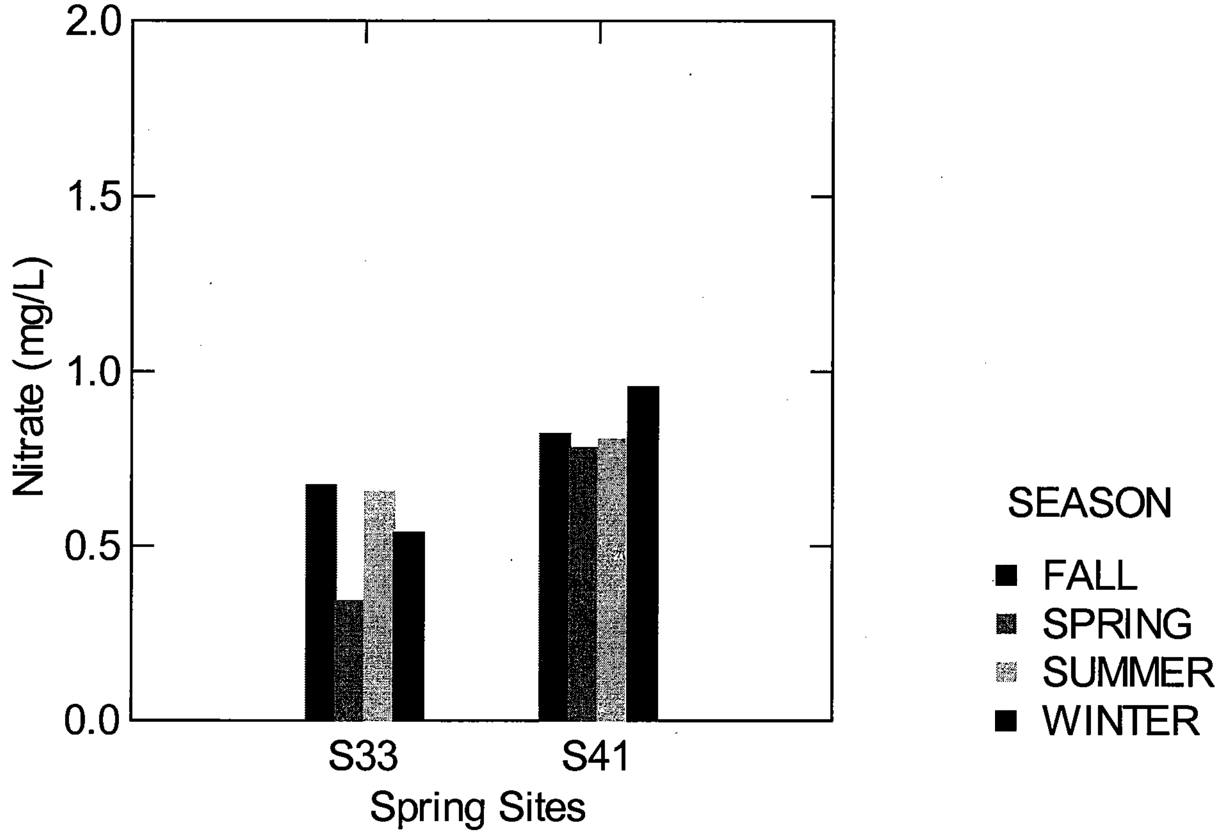


Figure 7. Seasonal nitrate concentrations (mg/L) from Gilbert Spring (S41) versus Mitch Hill Spring (S33).

Table 3. Seasonal means from physicochemical parameters collected from Mitch Hill Spring (S33) and Gilbert Spring (S41).

Site	Season	Temperature	Conductivity (mhos)	pH	Dissolved Oxygen	Turbidity	Fecal Coliform	Nitrates (NO_3)	Orthophosphates (PO_4)
S33	Spring	13.4	309.5	7.4	8.4	0.9	4.2	0.341	0.040
S33	Summer	15.6	357.9	7.3	7.1	1.1	10.2	0.655	0.025
S33	Fall	15.0	385.6	7.3	7.8	1.1	22	0.673	0.030
S33	Winter	13.2	335.0	7.5	9.3	1.0	3.9	0.537	0.025
S41	Spring	13.3	346.7	7.6	8.7	1.4	21.1	0.781	0.028
S41	Summer	17.0	385.2	7.6	7.6	1.0	60.2	0.805	0.034
S41	Fall	15.4	383.1	7.2	9.0	1.6	39.1	0.821	0.040
S41	Winter	11.4	327.2	7.6	10.3	1.3	47.5	0.954	0.032

Table 4. Seasonal means for physical habitat parameters collected from Mitch Hill Spring (S33) and Gilbert Spring (S41).

Site	Season	Depth (ft)	Velocity (ft/s)	Percent Canopy	Substrate Diversity	Substrate Size (mm)
S33	Spring	0.6	0.6	46	0.381	36
S33	Summer	0.5	0.8	65	0.371	42
S33	Fall	0.5	0.5	64	0.417	38
S33	Winter	0.6	1.4	45	0.379	37
S41	Spring	0.4	1.1	95	0.444	29
S41	Summer	0.2	0.6	100	0.393	18
S41	Fall	0.2	0.4	98	0.410	24
S41	Winter	0.3	0.8	80	0.499	18

Table 5. Bonferroni probability values from the Two-way ANOVA for Gilbert and Mitch Hill's macroinvertebrate communities. Dependent variables are based upon individual metrics and the integrative Index of Community Integrity (ICI). Unevenness in sample size is due to low value log transformations.

Dependent Variable	n	Seasons (degrees of freedom)	Sites (degrees of freedom)	Seasons*Sites
Taxa Richness	72	0.087(3)	**0.000**(1)	**0.024**
Species Diversity	72	0.068(3)	**0.000**(1)	**0.001**
% EPT (Log transformed)	65	**0.039**(3)	**0.006**(1)	**0.000**
% Diptera (Log transformed)	59	0.140(3)	**0.000**(1)	0.126
% Gammarus	72	0.087(3)	**0.000**(1)	0.062
%Agapetus (Log transformed)	50	0.440(3)	**0.006**(1)	0.068
IC1	23	0.360(3)	**0.006**(1)	0.555

Table 6. Spearman's results from spring systems exhibiting potential relationships with physical habitat.

Site	Dependent	Independent	Spearman's Coefficient	Probability Value
Mitch Hill Spring	Species Diversity	Substrate Diversity	+ 1.000	< 0.05
Mitch Hill Spring	% Diptera	Substrate Diversity	- 1.000	< 0.05
Mitch Hill Spring	% Gammarus	Substrate Diversity	+ 1.000	< 0.05
Mitch Hill Spring	% Agapetus	Substrate Size	- 1.000	< 0.05
Mitch Hill Spring	Nitrates	No biotic variable		
Mitch Hill Spring	Orthophosphates	No biotic variable		
Gilbert Spring	% Gammarus	Substrate Diversity	+ 1.000	< 0.05
Gilbert Spring	Nitrates	No biotic variable		
Gilbert Spring	Orthophosphates	No biotic variable		

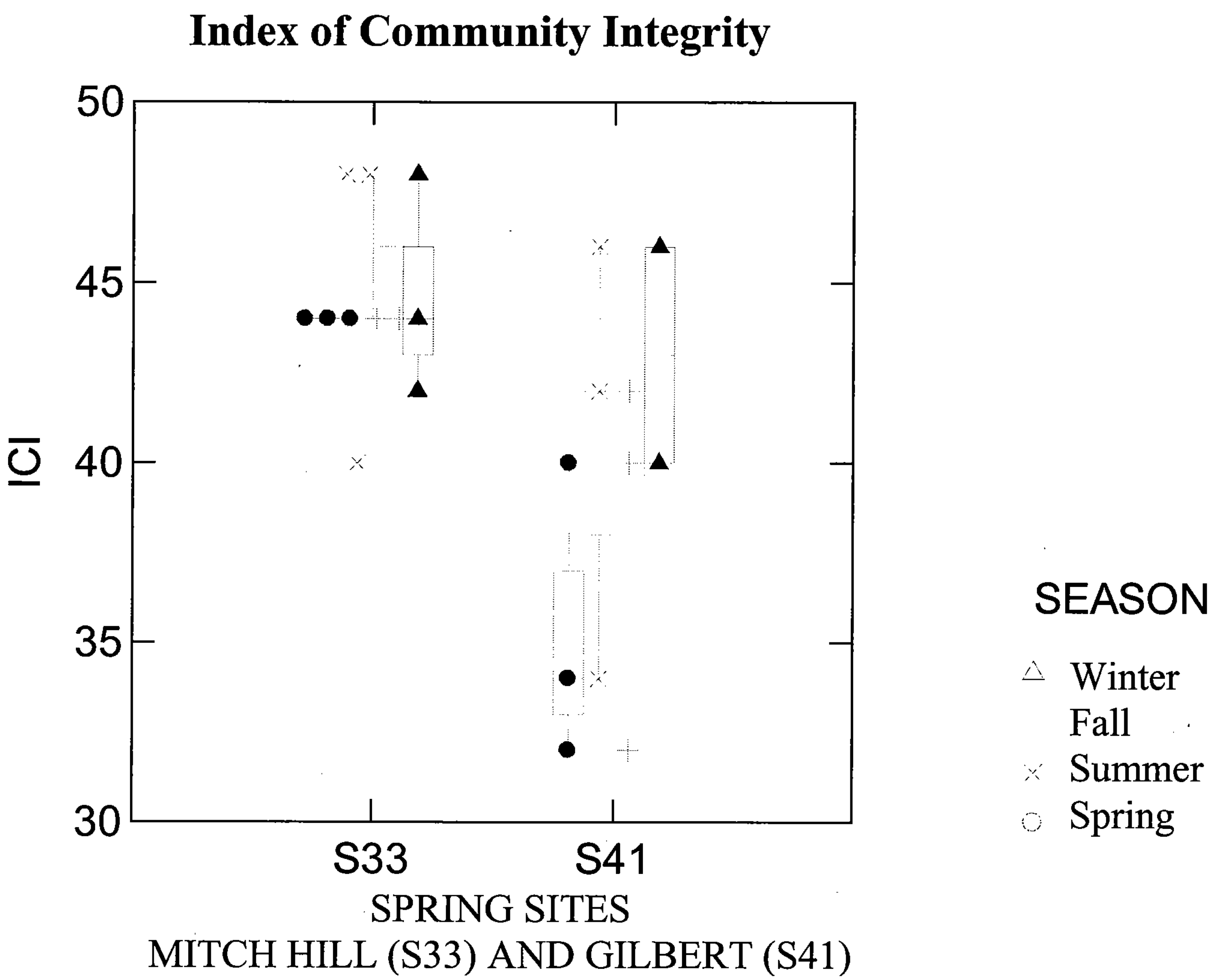


Figure 8. Comparison of Gilbert and Mitch Hill springs using the Index of Community Integrity. Box plot distributions are supplemented with symmetrical dot densities.

Discussion

Nutrient spiraling within streams describes the passage of an atomic element from an aquatic phase where it exists as a dissolved available nutrient, through its incorporation into living tissue (biotic phase) and possible passage through several links in the food chain before its eventual release into the water by excretion and/or decomposition (Allan, 1996). In most streams, biological uptake and assimilation of nutrients into organisms is accomplished primarily by autotrophs such as aquatic macrophytes, phytoplankton, and epilithic periphyton. Assimilation rates of dissolved nutrients by periphyton are influenced by the availability of nutrients and physical environmental parameters such as light, space, stream velocity, and water temperature. Aquatic community response to a moderate increase in nutrients will typically include an increase in periphytic density followed by shifts in macroinvertebrate community structure toward herbivory and the grazing functional feeding group (Allan, 1996).

Gilbert Spring was considerably higher in nutrients than Mitch Hill Spring, but no correlations between increasing nutrients and decreases in community indices or the ICI were found. The only herbivore that was consistently found between the two sites was *Agepetus*, which had higher percentages in Gilbert Spring (Table 2). Also of interest was the higher abundance of *Cheumatopsyche* in Gilbert Spring. *Cheumatopsyche* has a tolerance value of 6.6, and *Agepetus* has a value of 0, little or no tolerance of pollution (Southeast, Barbour et. al., 1999). This suggests that both pollution tolerant and intolerant genera of Trichoptera are existing within the system at Gilbert Spring and higher nutrients are not directly affecting the individuals or the community structure, although the evidence for the later is not conclusive. This result seems paradoxical when considering the higher nutrient concentrations found in Gilbert Spring.

The uptake of nutrients by autotrophs is controlled primarily by the availability of light, and other physical parameters (as discussed above) could be considered as secondary. Gilbert Spring was considerably more shaded than Mitch Hill Spring (Table 6). When considering the lack of direct sunlight and the shortness in system length, nutrient uptake may be limited in the Gilbert Spring system. This lack of ability of the aquatic community to fix energy biologically might explain the lack of community change based upon effects of poorer water-quality. Another explanation could be the collapsed sample size used within the statistical process of correlation. In order to couple the community indices with water-quality measurements the variability was reduced into a mean seasonal value. These seasonal mean values were then examined for variation relationship strengths with the individual indices and ICI. With the collapsed data set, the sample size was four per system, which requires a near perfect, succinct relationship in order to achieve statistical significance. This alone could explain the lack of correlative values between poor water-quality and declines in the macroinvertebrate community.

Taxa richness is typically expected to decrease within the macroinvertebrate community as general perturbation increases (Barbour et. al., 1999), and taxa richness was found to be higher in Gilbert Spring. This result should not be weighted too heavily when comparing the two systems based upon water-quality or the quality of the physical habitat. Generally, taxa richness was only higher at Gilbert Spring by one or two taxa, with few or only one individual representing that taxa group, depending upon season. Taxa richness within Gilbert Spring exhibited a positive relationship with increased bottom velocity and substrate size. This would indicate that a more suitable habitat was created by these two physical gradients. Another aspect that should be considered is the close proximity of the Buffalo River to the habitats within Gilbert Springs. The taxa richness found within the Buffalo River at the mouth of Gilbert Spring could be two or three times higher than found within Gilbert Spring. The closeness of the larger species pool to the community within Gilbert Spring could account for the higher taxa richness in Gilbert Spring than Mitch Hill Spring by physical setting alone. Evidence to support this explanation can be found in the presence of the caddisflies *Leptostoma* and *Chimarra* within Gilbert Spring. The collector-gatherers are commonly found within the river corridor, and were not expected to be found within a hypocrenal system.

Macroinvertebrate community diversity, as depicted by Simpson's index, was consistently lower in Gilbert Spring throughout all the seasons. No relationships were found with poorer water-quality, physical habitat, and macroinvertebrate community indices within Gilbert Spring. However, at a larger scale (the two systems combined, n = 72), potential relationships were found with increasing depth and substrate size. Perhaps, this suggests that Gilbert Spring was lower in diversity because it had more shallow habitats with smaller substrate sizes. This conclusion is logical based upon the location of the two systems. The entire length of the Gilbert Spring system lies within the flood plain of the Buffalo River and is subject to yearly inundation. With this seasonal flooding, sediments are deposited within the active hypocrenal channel covering the larger sediments and creating a physical habitat with smaller substrate types. Alternately, Mitch Hill Spring is located within a drainage basin system and channel morphology is maintained by flood events that occur within that drainage network. Mitch Hill Spring substrate size could also be increased by it's proximity to a gravel road were larger substrates are washed into the Mitch Hill system.

The Index of Community Integrity was especially designed for the Buffalo River and it's tributaries based upon seasonal water-quality values (i.e. nitrate concentrations) and macroinvertebrate collections over a period of 3 to 4 years. The ICI uses 10 metrics that examine the various characteristics of the macroinvertebrate community structure, as recommended by Barbour et al. (1999). Established ranges for each metric was normalized by assigning scores of 2 (for data of the poorest quality), 4, 6, and 8 (for data indicating various intermediate levels of water quality), and 10 (for data indicating best water quality). By normalizing in this manner, the effects of different measurement units and ranges in values can be eliminated, and no one metric is inherently more influential than any of the others. Once accomplished, scores for all metrics are summed and a total ICI score is generated.

Gilbert Spring was lower in ICI scores for all seasons. The ICI scoring system was created upon ranges in water-quality known to exist within the Buffalo River and it's tributaries, but the predictive properties for the individual metrics were originally designed to show changes within the macroinvertebrate community by general perturbation (Barbour, et al., 1999), which allows this biomonitoring program a dual utility. Statistical evidence indicates that the two communities are different based upon ICI scores, with Mitch Hill receiving the higher scores. Possible explanations for the difference between the communities could not be individually validated; however, the combination of poorer water-quality, differences in the quality of the habitat, and the physical setting (i.e. general perturbation) were most probably the factors that lead to Gilbert Spring having lower ICI scores.

Conclusions

Predictions of declining macroinvertebrate communities as a result of poorer water-quality within Gilbert and Mitch Hill Springs could not be validated. Gilbert Spring had higher nutrient values than did Mitch Hill Spring, but the disconnection of nutrients from the aquatic community due to physical shading within the habitat and low sample size most probably prevented correlative evidence from being elucidated. Based upon the ICI scoring system, Gilbert Springs had lower scores, which indicates that Gilbert Spring has a higher level of perturbation, but no individual factor was suggested by correlative efforts. Taxa richness was higher within the system of poorer water-quality. The increased taxa richness was attributed to the closeness of a larger species pool, and was not considered a product of the spring system. Macroinvertebrate community diversity was found to be much lower at Gilbert Spring, and a relationship was found that suggests diversity is negatively effected by smaller substrate sizes found within Gilbert Spring. This conclusion was supported by field observations and the positioning of the two spring systems as related to the Buffalo River's flood plain and the gravel road, which was responsible for the differences in substrate size and diversity between the two spring systems.

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